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MITOCHONDRIA-ASSOCIATED MEMBRANES (MAMs): OVERVIEW AND ITS ROLE IN PARKINSON'S DISEASE.

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ABSTRACT

Mitochondria-associated membranes (MAMs) are structures that regulate physiological functions between endoplasmic reticulum (ER) and mitochondria in order to maintain calcium signaling and mitochondrial biogenesis. Several proteins located in MAMs, including those encoded by *PARK* genes

and some of neurodegeneration-related proteins (Huntingtin, Presenilin, etc.) ensure this regulation. In this regard, MAMs alteration is associated with neurodegenerative diseases such as Parkinson (PD), Alzheimer (AD) and Huntington diseases (HD) and contributes to the appearance of the pathogenesis features, i.e. autophagy dysregulation, mitochondrial dysfunction, oxidative stress and lately neuronal death. Moreover, ER stress and/or damaged mitochondria can be the cause of these disruptions. Therefore, ER-mitochondria contact structure and function are crucial to multiple cellular processes. This review is focused on the molecular interaction between ER and mitochondria indispensable to MAM formation and on MAM alteration-induced etiology of neurodegenerative diseases.

KEYWORDS

Mitochondria, endoplasmic reticulum, neurodegenerative diseases, Parkinson's disease.

1.- ASSOCIATIONS BETWEEN MITOCHONDRIA AND OTHER ORGANELLES

Eukaryotic cells contain membrane-bound organelles with unique identities and specialized functions. Communication and cooperation between these organelles take place in order to maintain a variety of physiological functions. This communication requires the formation of specialized membrane microdomains at the contact sites, defining short distances between membranes to connect them, and allowing ions, metabolites and lipids exchange.

The best-characterized inter-organelle connection involves mitochondria and a specialized domain of the endoplasmic reticulum (ER), known as the mitochondria associated-membrane (MAM). ER-mitochondria contacts sites have been demonstrated to act as a signaling hub, playing a crucial role in lipid metabolism and calcium (Ca^{2+}) homeostasis, therefore regulating fundamental processes for the cell, such as mitochondrial morphology [1,2], ROS (reactive oxygen species) induced-cell stress [3], autophagy [4] and apoptosis (Reviewed elsewhere in [5]). Notably, several recent studies indicate that perturbations in the ER-mitochondrial network might underlie the pathogenesis of several neurodegenerative diseases [6,7] (see below). Therefore, the interest on mapping ER-mitochondria connections has risen considerably and current research is focused on determining their function, regulation and (patho) physiological consequences.

Mitochondria are known to communicate also with other organelles, although these interactions are less characterized at the molecular level than those with the ER. The recruitment of membranes to mitochondria is mediated through the formation of molecular tethers. Several protein complexes residing in mitochondria and other organelles have been identified as molecular tethers with new properties and functions. These proteins include various chaperones, protein kinases, mitochondria-shaping proteins, sorting proteins, etc. Contact sites between the endosomal system and mitochondria have been observed in reticulocytes [8]. Likewise, melanosomes-mitochondria contacts have also been described [9]. These particular contacts may be related to an additional metabolic demand, such as iron delivery or a higher

energetic demand, but it is possible that also applies to other mammalian cells. In *S. cerevisiae*, mitochondria are found to be closed to the contact of vacuoles by the vCLAMP (vacuole–mitochondrial patch) (Reviewed in [10]). Although interaction between mitochondria and lipid droplets or peroxisomes has been reported in some cell types [11,12], it remains unknown whether this interaction allows exchange of metabolites between them [13]. Recently, a mitochondria–plasma membrane tethering complex has been described in yeast, the Num1/Mdm36 (nuclear migration 1/mitochondrial distribution and morphology 36) complex [14,15]. This interaction has important functions during cell division and it supports mitochondrial division, however yeast homologs of the tethering complex are unknown in metazoan model systems.

2.- MITOCHONDRIA-ASSOCIATED MEMBRANES (MAMs)

Association between the ER and mitochondria was the first described inter-organelle contact. In early 60s, Copeland and Dalton demonstrated this connection by electron microscopy in the pseudobranch gland of a teleost [16]. Nearly twenty years later, several groups confirmed these findings using different microscopy techniques and co-sedimentation experiments [17,18]. Ten years later, fractionation studies by Jean Vance termed the biochemically distinct domains of the ER that are in close contact with mitochondria as MAM [19]. This and other earlier studies proposed MAMs ~~(mitochondria-associated membranes)~~ as a platform for lipid synthesis and transfer between ER and mitochondria [20-23]. Actually, the first proteins identified on the MAMs, PEMT2 (phosphatidyl ethanolamine methyltransferase 2) and PSS1/2 (phosphatidylserine synthase 1 and 2) [24,25], are associated with lipid metabolism, and FACL4 (fatty acid CoA ligase 4), which is involved in triacylglycerol synthesis, is currently considered one of the most reliable MAM marker proteins [26].

Subsequent studies by Rizzuto and co-workers showed that these structures are also involved in Ca^{2+} homeostasis, demonstrating Ca^{2+} transfer from ER to mitochondria in hotspots by live-cell imaging studies [27-29]. Besides, they showed that this exchange due to 5-20% of the mitochondrial surface is closely opposed (10-25 nm) to ER membranes [27]. Thus, MAMs can act as a Ca^{2+} signaling hub that accommodate the Ca^{2+} transfer from the ER to mitochondria in order to maintain cellular bioenergetics and mitochondrial dynamics, or to promote cell death [30,31]. The enrichment of the Ca^{2+} channel IP_3R (inositol-1,4,5-trisphosphate (IP_3) receptor) on the MAMs supports this model [32,33]. IP_3R contacts the outer mitochondrial membrane (OMM) protein VDAC1 (voltage-dependent anion channel isoform 1) through the molecular chaperone GRP75 (glucose-regulated protein 75), allowing Ca^{2+} transfer from the ER to mitochondria. Thus, it has been proposed that GRP75 acts as a bridge between them [29], strengthening the functional ER-mitochondria interaction by forming a tether complex with IP_3R (ER) and VDAC1 (mitochondria). Nevertheless, loss of IP_3R does not interfere in ER-mitochondria association, which argues against a physical tethering role for this Ca^{2+} channel [34].

ER-mitochondria contact sites are enriched with proteins that are involved in MAMs functions. In addition to the IP_3R-GRP75-VDAC1 complex, other MAMs proteins were proposed to act as molecular tethers as well, linking physically the ER to the mitochondria. In yeast, ER-mitochondria connection is mediated by the ER-mitochondria encounter structure (ERMES) complex, which is

composed of the ER protein Mmm1 (mitochondrial morphology protein 1) and the mitochondrial proteins Mdm10 (mitochondrial distribution and morphology protein 10), Mdm34, Mdm12 and Gem1 (GTPase EF-hand protein of mitochondria 1) [35]. This complex is also involved in lipid transfer and mitochondrial fission [36]. However, ERMES machinery has not ~~yet~~ been identified in mammalian cells yet. Electron microscopy and tomography studies have determined the presence of tethers of different sizes, suggesting that this contact may rely on different protein complexes in rat liver mitochondria and DT40 cells [34]. In addition, both rough and smooth ER form contacts with mitochondria in HT-1080 fibrosarcoma cells, indicating that selective recruitment of different domains of ER might depend on different tethering complexes and that several types of MAMs might exist [34,37]. MAMs proteins, such as PACS-2 (phosphofurin acidic cluster sorting protein-2) [38] and MFN2 (mitofusin 2) [39], have been implicated in the regulation of MAMs' formation and functions. PACS-2, a cytosolic sorting protein, regulates localization of the ER chaperone calnexin (CNX) and the Ca^{2+} channel TRPP2 (transient receptor potential protein 2) at MAMs [38,40,41]. These studies reported that PACS-2 down-regulation uncouples mitochondria from the ER. Nevertheless, it remains unknown whether this is a result of mitochondrial fragmentation, also observed in this study [38]. MFN2 is a dynamin-related protein that mediates, together with MFN1 (mitofusin 1) and OPA1 (optic atrophy 1), mitochondrial fusion. Furthermore, MFN2 is widely accepted as a regulator of the ER–mitochondria crosstalk by forming homotypic interactions and heterocomplexes with its homolog MFN1. However, this model has been recently challenged by different reports [37,42,43]. In fact, MFN2 ablation increased ER–mitochondria coupling, indicating that it plays a role as an ER–mitochondria tethering antagonist [37,42,43]. Finally, a novel tethering complex composed by the integral ER protein VAPB (vesicle-associated membrane protein-associated protein B) and the OMM protein PTPIP51 (protein tyrosine phosphatase-interacting protein 51) has been reported [44,45]. VAPB is enriched at MAMs and binds to PTPIP51 at MAMs. This interaction forms a molecular scaffold which tethers ER regions to mitochondria, regulating MAM functions, like the Ca^{2+} exchange between the two organelles [45,44,46].

3.- FUNCTIONAL ROLE OF THE MAMs

As we previously stated, MAMs are involved in several biological processes. Hereafter we will show what is known about its role and regulation in such processes. Table 1 summarizes all old and new findings related to MAMs biology.

3.1.- MAM is essential for mitochondrial morphology

Several proteins involved in mitochondrial movement along microtubules, such as dynein and kinesin, are tightly regulated by Ca^{2+}-sensors [47-49]. Mitochondrial movement is modulated by the cytosolic Ca^{2+} content. Its rise blunts the mitochondrial motility that can be recovered by EGTA (Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid) incubation or physiological Ca^{2+} level [49]. Evidence has demonstrated that reversible inhibition of mitochondrial motility, in Vasopressin (a Ca^{2+} mobilizing)-or Ionomycine (a Ca^{2+} ionophore)-treated cells, is related to the IP3-IP3R pathway-mediated Ca^{2+} [49]. Reduced mitochondrial motility induces an increase in their association to the

MAMs, subdomains of high Ca^{2+} release, thus enhancing their Ca^{2+} uptake and buffering capacity [49,50]. Miro family of proteins (Miro1 and 2) is one of the master regulators of mitochondrial motility, based on local Ca^{2+} levels. It has been shown that these proteins have an important role in tethering the mitochondria to the cytoskeleton by binding kinesin, thus leading to the movement of this organelle [51,52].

Beyond the regulation of mitochondrial motility, MAMs participate in the regulation of mitochondrial morphology [1]. Mitochondria are dynamic organelles, continuously undergoing fusion and fission. An appropriate balance between these two opposing processes is crucial for cell survival [53], as well as to maintain the shape, the size and the number of mitochondria [54,55]. Then, this balance is highly regulated and its defect leads to fragmented networks. Indeed, mitochondrial fragmentation is a process often stimulated in response to cellular stress [54]. The main mitochondrial fission and fusion proteins are dynamin-related proteins (DRP). Recruitment of DRP1 from the cytoplasm to the mitochondria is a key step in fission regulation. In 2011, Friedman and co-workers demonstrated that mitochondrial fission occurs at positions where ER tubules contact and constrict mitochondria. These constrictions would facilitate DRP1 recruitment [1]. Other proteins like Mff (mitochondria fission factor) and Fis1 (fission 1 protein) are important for DRP1 recruitment to mitochondrial fission sites and both proteins have also been localized to the MAM [56]. However, Mff localizes in a DRP1-independent manner to mitochondrial constrictions at sites of ER contact [56]. Recently, syntaxin 17 have been involved in mitochondria fission. This protein is present at the ER-mitochondria contact sites where interacts with Drp1 and modulates the mitochondria fission by regulating Drp1 activity and localization [57]. Therefore, all these findings suggest that the recruitment of the mitochondrial fission machinery to the MAMs is crucial for mitochondrial morphology.

3.2.- ER-mitochondria contact sites are involved in autophagosome biogenesis

Autophagy is a cellular response in which cytosolic components and damaged organelles, but also invading pathogens, are engulfed in double-membrane vesicles and delivered to lysosomes for their degradation [58]. The origin of the autophagosomal membrane is still controversial. Several cellular compartments have been pointed as potential membrane sources [59-62]. A recent study by the group of T. Yoshimori reported that ER-mitochondria contacts sites are involved in phagophore assembly. Upon autophagy induction, the pre-autophagosome marker ATG14L (autophagy-related 14-like) and DFCP1 (double FYVE domain-containing protein 1), a marker of the omegasome, relocalize to the MAMs to initiate autophagosome formation [4]. Moreover, autophagosome-formation marker ATG5 (autophagy-related 5), also shifts to the MAMs after starvation [4]. Interestingly, syntaxin 17 also participates in the autophagosome formation through its interaction with ATG14L promoting the recruitment of PI3P (phosphatidylinositol 3-phosphate) complex [57]. In contrast, the disruption of ER-mitochondria contact sites reduced the number of autophagosomes. Taken together, these results underscore the importance of MAMs in the biogenesis of autophagosome through the recruitment of autophagy-related proteins to the ER-mitochondria contact sites.

Besides its role in autophagosome biogenesis, MAM is important for mitophagy in yeast [63]. Mitophagy is a selective autophagy that removes damaged mitochondria [64]. Bökler and Westermann have described that mitochondria-ER contact sites are crucial for mitophagy. In yeast, mitochondrial ER tethering is mediated by ERMES complex. Defects in ERMES complex have strongly reduced rates of mitophagy [63]. Therefore, the mitochondria-ER contact sites have an active role in the initial steps of both autophagy and mitophagy in mammalian and yeast model, respectively.

The mTOR (mammalian Target of Rapamycin) kinase is also localized to the ER and mitochondria. The mTOR kinase is a negative regulator of autophagy and forms two functionally and structurally distinct complexes: mTORC1 and mTORC2 (mTOR complex 1 and 2) [65]. It has been reported that the localization of mTORC2 to MAMs is a growth factor-dependent signaling, regulating MAMs integrity and mitochondrial function via Akt [66]

3.3.- Ca^{2+} transport through MAM regulates important cellular processes.

Initial studies on intracellular Ca^{2+} signaling reported that ER and mitochondria could be functionally linked [67]. Although the ER is the major Ca^{2+} storage unit in the cell, mitochondria are also important Ca^{2+} reserve. However, resting mitochondrial Ca^{2+} concentrations are significantly low (in the range of hundreds of nM) and it is only when cytosolic Ca^{2+} concentrations are above µM that mitochondria can take up Ca^{2+} to reach the intramitochondrial levels of µM ranges [68].

Currently, we know that cyclical Ca^{2+} exchange between ER and mitochondria is essential for cellular viability. ER Ca^{2+} determines the correct activity of numerous ER-resident enzymes involved in the generation of secretory proteins, like CNX and calreticulin (CRT) [69]. Simultaneously, both chaperones interact with the MAM-enriched IP_3R and ER Ca^{2+} transport ATPase (SERCA) 2b, regulating ER–mitochondria Ca^{2+} flux [70,71]. Palmitoylated CNX interacts at the MAMs with SERCA2b to regulate Ca^{2+} signaling, whereas non-palmitoylated CNX mainly acts in the rough ER to mediate protein folding and quality control [72]. The ER protein Sig-1R (sigma-1 receptor) also localizes at the ER–mitochondria junction and can be considered as a *bona fide* MAM marker [73]. Sig-1R forms a Ca^{2+}-sensitive chaperone complex with BiP/GRP78 (binding immunogobulin protein/ glucose regulated protein 78) and prolongs Ca^{2+} signaling from the ER to the mitochondria by stabilizing IP_3R subunit 3 at MAMs [73]. Moreover, IP_3R interacts with VDAC1 at the OMM through GRP75, a chaperone located in MAMs, facilitating the Ca^{2+} uptake from ER to mitochondria [29]. VDAC1 selectively transfers apoptotic Ca^{2+} signals to mitochondria because of its Ca^{2+}-binding sites. This Ca^{2+}-permeability (increase of mitochondrial Ca^{2+}) favors VDAC1 oligomerization and triggers apoptotic cell-death by cytochrome *c* release [74]. In fact, GRP75 is a scaffold that would determine the ER-mitochondria contacts. The down-regulation of GRP75 abrogates the functional interaction between IP_3R and the mitochondria, altering the normal transfer of Ca^{2+} to the mitochondria [29]. Indeed, overexpression of VDAC1 has been shown to promote Ca^{2+} uptake into mitochondria [75]. Taken together, all these studies suggest that chaperone complexes at both the ER and the mitochondrion could coordinate Ca^{2+} regulation between these two organelles.

Furthermore, the transfer of Ca^{2+} from ER to mitochondria is crucial for mitochondrial function. ATP production is regulated by Ca^{2+} transfer from the ER as several mitochondrial dehydrogenases of the Krebs cycle are directly monitored by Ca^{2+} [76]. Increased ER-mitochondria communication is predicted to lead to mitochondrial Ca^{2+} overload, which could induce opening of the mitochondrial permeability transition pore, triggering apoptosis [5]. The most important Ca^{2+} channel responsible for Ca^{2+} release from ER to mitochondria is the IP_3R, which is highly concentrated in ER-mitochondrial contact sites. It has been shown that IP_3R activity is modulated by several interacting proteins [30,31]. In fact, it has been shown that cytochrome *c* binds to IP_3R channels during apoptosis, blocking the Ca^{2+}-dependent inhibition of IP_3R function and promotes apoptotic Ca^{2+} release [30]. Indeed, PTEN (phosphatase and tensin homolog) has also been reported to be located at the MAMs and enhances Ca^{2+} signaling to the mitochondria in situations of increased ER stress and pro-apoptotic signaling [77]. Moreover, during apoptosis, mitochondrial fragmentation increases due to the recruitment of the fission protein DRP1 to the OMM. In this particular location, DRP1 also stimulates BAX (Bcl2-associated X protein, a pro-apoptotic OMM protein) to form oligomeric pores that cause OMM permeabilization (OMMP), causing the release of cytochrome *c* and other pro-apoptotic factors to the cytosol [55]. Conversely, when mitochondrial fusion is increased, apoptosis is attenuated.

Interestingly, the disruption of MAMs by PACS-2 depletion induced apoptosis [38]. PACS-2 is a MAM-associated multi-functional sorting protein required for extrinsic and intrinsic apoptosis. The absence of PACS-2 was reported to induce UPR (unfolded protein response) and mitochondria fragmentation and BAP31 (B-cell receptor associated protein 31)-mediated apoptosis [38].

Ca^{2+} depletion and accumulation of unfolded or misfolded proteins in the ER lumen elicit ER stress. Under these conditions, three proteins located in the ER membrane PERK (protein kinase RNA-like ER kinase) [78], IRE1 (inositol-requiring protein 1) [79] and ATF6 (activating transcription factor 6) [80] act as specialized stress sensors, starting UPR. These proteins are able to activate signaling pathways which involve ER-resident chaperones, including BiP/GRP78 and the lectins CNX and CRT [81], to mitigate and buffer ER stress, and finally recover the ER functionality. Moreover, protein folding requires high levels of energy, thus ER homeostasis is tightly regulated. This specialized ER-redox environment is maintained by oxidoreductase enzymes. Some members of this group are PDI (protein disulfide isomerase) and Ero1 (ER oxidoreductase 1) which are implicated in the formation of disulfide bonds [82].

During early phases of ER stress, ER–mitochondrial connection is enriched in the perinuclear zone of cells [83]. In this cellular context, it would be feasible that connections between ER and mitochondria participate in the regulation of ER stress.

Indeed, it has been shown that PERK is enriched at the MAMs, being required for the regulation of inter-organellar communication during ROS-induced cell death [84]. Furthermore, ~~genetic ablation of~~ *PERK* deficiency decreased mitochondrial Ca^{2+} uptake, conferring mitochondrial protection against ER stress-mediated apoptosis by the reduction of caspase activation and cytochrome *c* release [84]. A recent study by the group of Zorzano reported that the mitochondrial protein MFN2 physically interacts with PERK and this binding is required for the proper regulation of cellular homeostasis upon ER stress [85].

BiP/GRP78, apart from its role in protein folding, is involved in Ca^{2+} buffer in the ER lumen [86]. In this regard, it has been shown that BiP forms a complex with the ER protein Sig-1R, to control the mitochondrial Ca^{2+} signaling at MAM. These authors observed that ER stress induction by TG (thapsigargin, an inhibitor of the sarco-endoplasmic reticulum Ca^{2+}- pump) treatment induces dissociation between BiP and Sig-1R, and the translocation of Sig-1R from the MAM to other ER regions [87]. As mentioned before, another ER-resident chaperone, CNX, is also enriched at the MAM after palmitoylation [88], suggesting that this mechanism could mediate protein re-localization to these cellular hubs. Related to this, it has been shown that the cytosolic sorting protein PACS-2 is also responsible for the CNX enrichment at the MAMs [89].

Other studies have revealed that Ero1α, one of the two mammalian isoforms of Ero1, accumulates at the MAMs during oxidizing and normoxic conditions [90] and modulates Ca^{2+} fluxes [91]. Nevertheless, we cannot exclude that this Ero1α enrichment is linked to other specific functions at the ER-mitochondria interface.

Taken together, all these findings demonstrate that MAMs regulate the Ca^{2+} flux between the ER and mitochondria through the accumulation of Ca^{2+} signaling proteins in the MAMs compartment. Higher Ca^{2+} transfer from ER to mitochondria mediates mitochondrial Ca^{2+} overload that induces mitochondria dysfunction and apoptosis. However, the disruption of ER- mitochondria association decreases the flux of Ca^{2+} between these two organelles and reduces cell viability. Moreover, perturbations of ER-mitochondria contact sites induce ER stress. Therefore, MAMs play a key role of Ca^{2+} signaling inducing processes such as ER stress and apoptosis.

3.4.- Role of MAM in inflammatory response induced by ROS

In 2011, the group of Tschopp observed that ROS promoted NLRP3 (Nod-like receptor family, pyrin domain containing 3) inflammasome activation, linking ER–mitochondria interface and inflammation [92].

The inflammasome is a multiprotein complex of the innate immune response that regulates the activation of caspase-1 and triggers the processing and maturation of proinflammatory cytokines such as IL-1β (interleukin-1β) and IL-18 [93,94]. There is a whole family of inflammasomes, NLRP3 inflammasome is the most thoroughly characterized and comprises the NLRP3 protein, the adapter apoptosis-associated speck-like protein (ASC) and pro-caspase-1. [95] Upon MSU (monosodium urate) and, in particular, nigericin addition, NLRP3 co-localizes with ASC proteins at the MAM fraction and ASC translocation to the MAMs seems to be NLRP3-dependent [92].

The generation of mitochondrial ROS appears to be common to many activators of the NLRP3 inflammasome [96]. As mentioned before, VDAC1 are important regulators of mitochondrial metabolic activity through the uptake of Ca^{2+} into the mitochondria from MAMs. It has been described that VDAC1 is ultimately required for mitochondrial ROS production. In this regard, the downregulation of VDAC1 significantly impairs NLRP3 inflammasome activation [92]. The critical role of MAMs in NLRP3

inflammasome activation is still unclear. However, all these findings suggested the crucial role of the MAMs in the coordination of cell non-autonomous functions, like inflammation.

Moreover, another NLRP3 binding partner, TXNIP (Thioredoxin-interacting protein), activates the NLPR3 inflammasome under mitochondrial oxidative stress condition to mediate inflammatory response in primary rats hepatocyte [97] as well as in THP1 macrophage cells [92]. In fact, TXNIP is mainly expressed in the nucleus under normal physiologic condition. In H_2O_2-treated β pancreatic cells, it has been demonstrated the translocation of TXNIP from the nucleus to mitochondria. This translocation makes cells vulnerable to oxidative stress as TXNIP binds to the thioredoxine-2 [98]. Of note, NLRP3 is found to reside principally in ER together with the CRT ER marker. NLRP3 moves on the ER-mitochondria contact site resulting from inflammasome activation by MSU or nigericin treatment [92]. Thus, it thought that ROS production increases TXNIP expression that provokes NLRP3 inflammasome activation. Interestingly, antioxidant treatments mitigate NLPR3 activation-induced ROS-TXNIP pathway [97]. These results suggest that MAMs have an important role in initiating the inflammatory response to oxidative stress.

3.5.- Regulation of antiviral signaling by ER-mitochondria contact sites.

Cytokines, and their corresponding cellular receptors, play a role during the inflammasome activation, but are also involved in the antiviral response. Basically, the first line of defence of the innate immune system is the pathogen recognition by PRR (pattern recognition receptor) proteins, which detect specific molecular signs of the pathogen, called PAMP (pathogen-associated molecular pattern) and activates the antiviral signaling pathway. Intracellular double stranded RNA (dsRNA) is a viral replication signature and is considered as a PAMP.

The RNA helicase RIG-I (retinoic acid inducible gene I) recognizes dsRNA, triggering innate antiviral response [99]. RIG-I also contains 2 CARDs (caspase activation and recruitment domains) in the N-terminal region, which are accessible for Lys63-linked ubiquitination by TRIM25 (tripartite motif containing 25) upon interaction of non-self-cytosolic RNA [100]. Once RIG-I is activated, it triggers host response signaling, including type I interferon (IFN) expression [101]. During virus infection, RIG-I senses dsRNA as non-self and is recruited to its adaptor protein MAVS (mitochondrial antiviral-signaling protein; also called VISA, IPS-1 or Cardif), initiating the cellular antiviral response [102]. Interestingly, this transmembrane protein is localized to both peroxisomes and mitochondria [103,104], as well as to the MAMs [105]. The specific location of the complex RIG-I and MAVS at the MAM is driven by the tethering factor MFN2 [105].

In addition, Hepatitis C virus (HCV) is sensed by RIG-I [106], however this virus uses its NS3/4A protease to cleave MAVS, avoiding antiviral induction [107]. Interestingly, NS3/4A is able to process MAM-associated MAVS, but not mitochondrial-associated MAVS [105], indicating that MAVS subpopulation located at this certain cellular hub is critical for the antiviral signaling against HCV [108]. Recently, the group of Michael Gale Jr. has identified, by proteomic analysis, MAM localization of new proteins during RNA virus replication, such as RAB1B (member RAS oncogene family 1B), VTN

(vitronectin) and LONP1 (Lon Peptidase 1) [109]. Therefore, the importance of the MAMs in the innate immune response to RNA viruses would rely on its function as a key cellular platform, contributing to the dynamic re-localization of protein complexes to initiate antiviral signaling.

3.6.- Involvement of MAM in lipid metabolism

Lipid synthesis, including triacylglycerol, PC (phosphatidylcholine) and PE (phosphatidylethanolamine), requires enzymatic activity associated with both the ER and mitochondria. Phosphatidylserine (PS) is formed from PC by PSS1 in MAM and is converted to PE by PS decarboxylase in mitochondria. Interestingly, one of the final enzymes implicated in PC synthesis, PEMT2 (phosphatidylethanolamine N-methyltransferase 2) [110], was found to be restricted to the MAMs [25]. Moreover, other studies demonstrated that PC synthesis requires a tightly regulated lipid transfer between the ER and mitochondria, thus confirming earlier hypothesis [111]. Acyl-CoA: diacylglycerol acyltransferase 2 (DGAT2) is also located at ER-mitochondrial contact site. It catalyzes triacylglycerol synthesis and promotes lipid droplet formation [112]. DGAT2 has a mitochondrial-targeting signal that promotes its association with mitochondria [112]. However, the MAM enrichment mechanism for PEMT2 and PSS1 is still not understood.

MAM contains other lipid metabolism enzymes identified as MAM markers such as FACL4, which mediates the ligation of fatty acids to coenzyme A [113] and Acyl-Coenzyme A:cholesterol acyltransferase 1 (ACAT1/SOAT1). The enzymatic activity of ACAT1/SOAT1 is the most enriched in this specific cellular location [26], acting as a multimembrane-spanning enzyme that catalyzes the production of cholesterol esters, which are subsequently incorporated into lipid droplets.

Therefore, the transport of lipids between the ER and mitochondria, and the accumulation of lipid metabolism enzymes on the MAMs are crucial for lipid metabolism [26]

4.- ROLE OF MAMS IN SEVERAL NEURODEGENERATIVE DISORDERS

As discussed above, MAMs play a crucial role in several signaling pathways and metabolic processes, including lipid metabolism, autophagy regulation and mitochondrial physiology, among others. Many of these functions are disrupted in several neurodegenerative diseases. Indeed, alterations in ER-mitochondria associations have been reported in Parkinson's disease (PD) and other neurodegenerative disorders that are deeply commented below (Figure 1). Most of the findings related to the role of MAMs in neurodegenerative diseases are included in Table 2. Neurodegenerative diseases are characterized by the accumulation of proteins that are somehow the main inducer of inflammatory response and consequently ER stress. It is well known that ER is required for the MAM formation and its morphological alteration can affect MAM function. Firstly, it is difficult to speculate whether MAM dysregulation is the cause or consequence of neurodegenerative disorders. However, it is clear that such dysfunction participates in the acceleration of neuronal death.

4.1.- Alzheimer's disease (AD)

The AD-related proteins PS1 and PS2 (Presenilin-1 and -2) are highly located in the MAMs. PS1 and PS2 are components of the γ-secretase complex that processes the amyloid precursor protein (APP), releasing the toxic β-amyloid peptide (Aβ) [114]. Their localization at MAM may explain the mitochondrial oxidative damage associated with abnormal APP processing and Aβ accumulation in mitochondria. In this sense, mutations in PS1, PS2, and APP have been shown to upregulate ER-mitochondria associations and intensify mitochondrial Ca^{2+} uptake. In fact, in both familial and sporadic AD, the interactivity of ER-mitochondria is upregulated, as well as MAMs function [6]. Lines of evidence point to the increasing expression of MAM-associated proteins in postmortem AD and in a $APP_{swe/lon}$ mouse model of AD, and protein upregulation, including PACS-2, VDAC1 and IP_3R, is inherent to new ER-mitochondria contacts [7]. However, this overexpression is not observed in an APP_{arc} mouse model, possibly because of the milder pathology of this model compared to the other one [7]. As the latter model has an intra Aβ mutation instead of mutations in β and γ cleavage sites of the former, this finding suggests an interaction between Aβ proteins and MAMs that should be further addressed. In spite of this upregulation of MAMs in AD patients, decreased levels of the ER protein Sig-1R were reported in a postmortem hippocampus of AD patients [115].

4.2.- Amyotrophic lateral sclerosis (ALS)

Sig-1R is an ER chaperone protein, located in MAM and mitochondria, as it was previously mentioned. ER stress causes a shift of Sig-1R from MAM [116] toward ER region. Loss of function (by gene mutation or down-regulation) of Sig-1R is responsible for several forms of ALS. The gene mutation E102Q, located in the transmembrane domain of the corresponding protein, was found in a juvenile ALS form [117].

As Sig-1R, the ALS-related VAPB protein is also localized in the MAMs. As mentioned before, VAPB interacts with the OMM protein PTPIP51 (protein tyrosine phosphatase-interacting protein-51) [45]. Mutated VAPB-P56S has been found to increase its affinity to bind to PTPIP51 and consequently, increases Ca^{2+} transfer into mitochondria. VAPB mutation could also affect lipid transfer due to its ability to interact with other ER proteins, like OSBP (oxysterol binding protein). Indeed, it was shown that mutant VAPB prevents the ER localization of OSBP, as well as reduction of phosphoinositides [118]. Furthermore, ALS-associated TDP-43 (TAR DNA-binding protein-43) reduces the VAPB-PTPI51 interaction by GSK-3β (glycogen synthase kinase-3 beta) activation, disrupting ER-mitochondria association [45].

4.3.- Huntington's disease (HD)

Huntingtin protein, whose polyglutamine repeats mutation is implicated in Huntington's disease (HD), has been found to be an ER-associated protein [119]. Its presence is important for ER morphology, but overexpression of mutant huntingtin elicits cell death in PC6.3 neuronal cell by generating ER stress [120]. While ER stress or UPR is activated, huntingtin protein shuttles from ER to the nucleus. Polyglutamine-expanded huntingtin triggers ER stress because of protein aggregates formation and

upregulates BiP/GRP78, CHOP (C/EBP homologous protein), PDI and Sig-1R protein levels [116]. Although Sig-1R is important for UPR regulation during ER stress, it is shown to be decreased in several neurodegenerative disorders, including AD, HD and Parkinson's disease (PD) [121,122]. The reason of such reduction may be due to its capacity to bind protein aggregates in neurodegenerative diseases [116]. Interestingly, in a HD cell model, Sig-1R levels were reestablished by the agonist receptor and, subsequently, mutant huntingtin toxicity was prevented [116]. Indeed, mutant huntingtin impairs the ER-associated degradation (ERAD) and vesicle trafficking [123], that favor proteins inclusions. As long as ER stress is activated, Ca^{2+} transfer from the ER to mitochondria is deregulated through MAM interface.

4.4.- Neuronal ceroid lipofuscinosis (NCL)

Ceroid-lipofuscinosis neuronal 3 (CLN3) is a lysosomal transmembrane protein whose mutation is implicated in the infantile neurodegenerative disease, neuronal ceroid lipofuscinosis (NCL) [124]. It was reported that the yeast homolog of *CLN3*, *BTN1* (battenin CLN3 family protein), plays a crucial role in phospholipids distribution between ER and mitochondria. *BTN1* deletion decreases PS transfer from ER to mitochondria as well as impairs PE levels in the MAMs [125].

5.- INVOLVEMENT OF MAMs IN PARKINSON'S DISEASE (PD)

Mitochondria dysfunction has been proposed as one of the major contributor in the pathogenesis of the most common neurodegenerative diseases [126]. Nowadays, PD-related genes have already been involved in mitochondrial regulation and PD-associated mutations in these genes have been related to mitochondrial dysfunction. SNCA (α-synuclein) is linked to familial (autosomal dominant early-onset PD) as well as sporadic PD. SNCA aberrant aggregation in oligomers has been shown to be toxic. The effect of SNCA oligomerization on mitochondria has been studied *in vitro* and *in vivo* and its effects include inhibition of mitochondrial complexes, as well as increase in the fragmentation of this organelle (Reviewed elsewhere in [127]). Moreover, other two PD-related proteins, PINK1 (PTEN-induced kinase 1) and Parkin are also involved in mitochondrial regulation, controlling its degradation by mitophagy, a selective type of autophagy for degradation of old/damaged mitochondria [127]. Also, cell lines derived from PD patients with mutated forms of *DJ-1*, as well as M17 neuroblastoma cells overexpressing those mutants, display aberrations in mitochondrial morphology [128,129]. Finally, fibroblasts derived from PD-patients with mutations in *LRRK2 (*leucine-rich repeat kinase 2*)*, *PINK1* and *Parkin* show clear mitochondrial abnormalities [130].

As well as the previous organelle, there is a strong evidence supporting the idea that ER stress may be an additional mechanism of pathogenesis in PD and other chronic diseases [131]. In this regard, *SNCA* overexpression is able to induce ER stress and promote UPR in rats [132], but also in mice overexpressing wild type *SNCA* and human pathogenic variants, such as A53T [133]. LRRK2 has been recently involved in anterograde Golgi transport, and its PD-related mutation R1441C disturbs ER-Golgi function [134].

To date, there is a little-known about the role of PD-related proteins in the interaction between ER and mitochondria. Nonetheless, in this review, the implication of the most studied *PARK* genes related to MAM structure or function is highlighted:

5.1.- α-synuclein (*SNCA*)

SNCA (codified by *PARK1* and *PARK4* genes) is a small soluble protein highly expressed in nervous tissues. SNCA aggregates were identified as a major component of Lewy bodies in PD. The majority of SNCA is found in soluble form in the cytoplasm. However, under pathologic conditions, SNCA binds to lipid membranes and may alter its conformation. A subpopulation of SNCA is present in MAM meanwhile this relationship is perturbed during pathogenic conditions. Mutated forms of this protein, such as A30P and A53T, showed lower association with MAMs in M17 cells and in brain of transgenic mice overexpressing each of these mutant proteins because of different reasons. A30P mutation alters the interaction between SNCA and rafts, meanwhile A53T mutation results in a lower amount of SNCA, and, consequently, lower association [135]. Besides, a decrease of ER-mitochondria tethering, a mitochondrial fragmentation has been found in these *in vitro* models [135,136]. In addition, SNCA is involved in Ca^{2+} homeostasis in HeLa cells since its silencing or its aggregation diminish Ca^{2+} uptake to the mitochondria [137]. Moreover, the presence of SNCA and its role in MAMs may influence in the cholesterol regulation alterations in PD as a consequence of MAMs dysfunction caused by SNCA mutations [135].

5.2.- Parkin and PINK1

Parkin (codified by *PARK2* gene) has proved to be a key protein in mitochondrial homeostasis, as mentioned above, in association with PINK1 (codified by *PARK6* gene) [127]. Impairment of Parkin function or activity contributes to neuronal loss in PD models [138]. Under ER stress, Parkin is upregulated by the ER stress pathway (PERK/ATF4). This overexpression prevents the ER stress-induced mitochondrial damage and confers to the protein Parkin a protective role [139]. This evidence has been supported by several cell lines (SH-SY5Y, HEK293T, MEFs) exposed to different ER stressors (TG, L-histidinol, Tunicamycin) [139]. Interestingly, neuronal toxins, such as MPP^+ and 6-OHDA, activate the ER-stress response through the activation of PERK and the phosphorylation of eIF2α (eukaryotic initiation factor 2α). PERK-elF2α pathway provokes the expression of the transcription factor ATF4 (activating transcription factor 4) in neuronal PC12 cells [138]. ATF4 possesses a binding site in *Parkin* promotor and enhances its mRNA expression as well as its protein level [139]. Nevertheless, Parkin upregulation does not modulate ER stress but do maintain mitochondrial integrity [139]. Although mitochondrial toxins increase Parkin mRNA expression level, its endogenous protein level seems to be reduced by proteasomal degradation. Thereby, overexpression of ATF4 maintains the Parkin protein level reduced by toxins [138]. In this line, *Parkin* overexpression results in a better maintenance of ER-mitochondria contact sites and Ca^{2+} homeostasis in HeLa cells [140].

5.3.- DJ-1

DJ-1 is a protein with a wide variety of functions; its plays an essential role as a redox-dependent chaperone, as well as an autophagy modulator. ~~among others~~ [141]. *DJ-1* (*PARK7*) loss-of-function mutations are associated with rare forms of inherited early-onset PD and result in oxidative stress-induced mitochondrial dysfunction [142,143]. The role of DJ-1 in Ca^{2+} uptake was studied in 2013 by Ottolini and co-workers. Their results showed that *DJ-1* downregulation reduces Ca^{2+} transfer from ER to mitochondria, promoting morphological changes in mitochondria and decreasing mitochondrial Ca^{2+} uptake. Therefore, DJ-1 exerts a cytoprotective role maintaining Ca^{2+} transfer through tethering ER to mitochondria. Moreover, its overexpression can counteract p53 effects on mitochondrial deregulation, suggesting a great importance of the contact between these organelles to preserve normal physiology [144].

6.- CONCLUSIONS AND FUTURE PERSPECTIVES

Overall, ER-mitochondria contacts are critical in many cellular processes and signaling pathway. These contact sites between ER and mitochondria involved proteins that ensure MAMs structure and/or function. MAM formation regulates Ca^{2+} homeostasis by a reversible mitochondrial movement inhibition. Mitochondria are distributed in an space-temporal manner throughout the ER in Ca^{2+}-enriched domains and assure Ca^{2+} uptake [49]. In this regard, we speculate that uncoupling mitochondria failed to participate in MAM formation. Thus, for preventing the clearance of damaged mitochondria, autophagy/mitophagy inhibition can disrupts the ER-mitochondria tether. Although MAM is important to autophagosome formation, it probably exist a bidirectional link between autophagy and MAM. Of note, mitochondria contribute to the regulation of ER stress by Ca^{2+} uptake. Nevertheless, Ca^{2+} overload in mitochondria triggers cell death by cytochrome *c* release and caspases activation. In AD [6] and HD [116], mitochondrial Ca^{2+} uptake is heightened due to the upregulation of MAM proteins while decrease in some models of ALS and PD [144]. It is still unclear how MAM function is either upregulated or disrupted in neurodegenerative diseases.

Compelling evidence support the hypothesis that MAMs and their deregulation may play an important role in PD pathogenesis, even as an early event [145]. The relationship between PD-related proteins, such as SNCA, Parkin and DJ-1, and the ER-mitochondria axis, and how their pathogenic mutations alter these interactions, strongly support this idea. In this respect, these findings highlight the therapeutic potential of targeting the ER-mitochondria axis. Future studies to determine if other PD-related genes, such as *LRRK2* or *GBA* (glucoside beta acid), may interfere with the regulation of the mitochondria-ER network would be useful to a better understanding of a possible general role of PD-related proteins in the MAMs, allowing the development of specific therapeutic approaches. Future works in this field will provide a deeper view of the mechanisms underlying the regulation of these critical hubs, and how pathogenic situations affect MAMs stability.

ACRONYMS

Aβ: amyloid beta.

ACAT1/SOAT1: Acyl-Coenzyme A: cholesterol acyltransferase 1.

AD: Alzheimer's disease.

ALS: Amyotrophic lateral sclerosis.

APP: amyloid precursor protein.

ASC: apoptosis-associated speck-like protein.

ATF6: activating transcription factor 6.

ATG14L: autophagy-related 14-like.

ATG5: autophagy-related 5.

BAX: Bcl2-associated X protein.

BiP/Grp78: binding immunoglobulin protein/glucose regulated protein 78.

BTN1: battenin CLN3 family protein.

Ca^{2+}: calcium.

CARDs: Caspase activation and recruitment Domains.

CHOP: C/EBP homologous protein.

CLN3: ceroid-lipofuscinosis neuronal 3.

CNX: calnexin.

CRT: calreticulin.

DGAT2: Acyl-CoA: diacylglycerol acyltransferase 2

DRP1: dynamin-related protein 1.

dsRNA: double-stranded RNA.

EGTA: Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid

eIF2α: eukaryotic initiation factor 2α

ER: endoplasmic reticulum.

ERAD: ER-associated degradation.

ERMES: ER-mitochondria encounter structure.

Ero1α: ER oxidoreductase alpha.

FACL4: fatty acid CoA ligase 4.

Fis1: fission 1 protein.

GBA: glucosidase beta acid.

Gem1: guanosine tri-phosphatase (GTPase) EF-hand protein of mitochondria 1.

GRP75: glucose-regulated protein 75.

GRP78: glucose-regulated protein 78.

GSK-3β: glycogen synthase kinase-3 beta.

HCV: Hepatitis C virus.

HD: HuntingtonHuntington's disease.

IFN: interferon.

IL-1β: interleukin-1 β.

IL-18: interleukin-18.

IP_3R: inositol-1,4,5-trisphosphate (IP_3) receptor

IRE1α: inositol requiring enzyme 1 alpha.

LONP1: Lon peptidase 1.

LRRK2: leucine rich repeat kinase 2.

MAM: mitochondria associated-membrane.

MAVS: mitochondrial antiviral-signaling protein.

Mdm10: mitochondrial distribution and morphology protein 10.

Mdm12: mitochondrial distribution and morphology protein 12.

Mdm34: mitochondrial distribution and morphology protein 34.

Mff: mitochondrial fission factor.

MFN1: mitofusin 1.

MFN2: mitofusin 2.

Miro: mitochondrial Rho GTPase.

Mmm1: mitochondrial morphology protein 1.

MSU: monosodium urate.

mTOR: mammalian Target of Rapamycin

mTORC1: mTOR complex 1.

mTORC2: mTOR complex 2.

NCL: neuronal ceroid lipofuscinosis.

NLRP3: NOD-like receptor protein 3 o Nod-like receptor family, pyrin domain containing 3.

NOD: nucleotide binding oligomerization domain

OMM: outer mitochondrial membrane.

OMMP: outer mitochondrial membrane permeabilization.

OPA1: optic atrophy 1.

OSBP: oxysterol binding protein.

PACS-2: phosphofurin acidic cluster sorting protein-2.

PAMP: pathogen-associated molecular pattern.

PC: phosphatidylcholine.

PD: Parkinson's disease.

PDI: protein disulfide isomerase.

PEMT2: phosphatidyl ethanolamine methyltransferase 2.

PERK: protein kinase RNA (PKR)-like ER kinase.

PINK1: PTEN-induced putative kinase 1.

PRR: pattern recognition receptor.

PS: phosphatidylserine.

PS-1: Presenilin-1

PS-2: Presenilin-2.

PSS1/2: phosphatidylserine synthase-1 and -2.

PTEN: phosphatase and tensin homolog.

PTPIP51: protein tyrosine phosphatase-interacting protein 51.

Rab1B: member RAS oncogene family 1B.

Rab32: member RAS oncogene family 32.

RIG-I: retinoic acid inducible gene I.

ROS: reactive oxygen species.

SERCA: sarcoendoplasmic reticulum Ca^{2+} Transport ATPase.

Sig-1R: sigma-1 receptor.

SNCA: α-synuclein.

TDP-43: TAR DNA-binding protein-43.

TG: thapsigargin.

TRIM25: tripartite motif containing 25.

TRPP2: transient receptor potential protein 2.

TXNIP: thioredoxin interacting protein.

UPR: unfolded protein response.

VAPB: vesicle-associated membrane protein-associated protein-B.

vCLAMP: vacuole-mitochondrial patch.

VDAC1: voltage dependent anion channel.

VTN: vitronectin.

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Figure 1. **Mitochondrial-associated ER-membranes (MAMs) and neurodegenerative disorders. A.** Schematic illustration of structural MAM's network proteins **B.** Localized events in MAMs that contribute to the pathogenesis of neurodegenerative diseases, *i.e.*, elevated activity of presenilin-1 (PS1) in MAMs in connection with the Alzheimer's disease; Loss of function of sigma-1 receptor (Sig-1R) defective or interaction between vesicle-associated membrane protein-associated protein B (VAPB) and protein tyrosine phosphatase-interacting protein 51 (PTPIP51) in relation to Amyotrophic lateral sclerosis; mutations in huntingtin (HTT) linked to Huntington's disease; or dysfunction on proteins present in MAMs (as α-synuclein, Parkin or DJ-1) correlates also with the Parkinson's disease. Aβ, amyloid-β; Ca^{2+}, calcium; ER, endoplasmic reticulum; GPR75, glucose-regulated protein 75; IP3R, inositol-1,4,5-trisphosphate (IP3) receptor; MFN, mitofusin; PACS-2, phosphofurin acidic cluster sorting protein-2a-syn; poly-Q, polyglutamine; TRP, transient receptor potential channel; VDAC1, mitochondrial voltage-dependent anion channel isoform 1.